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Alterations in Catecholamine Neurons of the Locus Coeruleus in Senile Dementia of the Alzheimer Type and in Parkinson's Disease With and Without Dementia and Depression

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ABSTRACT

The present study provides qualitative and quantitative investigations of the norepinephrine (NE) neurons in the locus coeruleus (LC) in two neurodegenerative disorders, the senile dementia of the Alzheimer type (SDAT) and Parkinson's disease (PD). The group of PD subjects was subdivided into cases without dementia (P−D), cases with dementia, L-dopa responsive (P+D), and cases with fulminant dementia whose motor disorder symptoms were L-dopa nonresponsive (P+D/L-dopa non-responsive). NE neurons were demonstrated by immunocytochemistry against tyrosine hydroxylase (TH). Quantitations of neuronal parameters and cell numbers and three-dimensional reconstructions of the LC were carried out with a computer-assisted system.

In SDAT cases, the rostrocaudal LC length (13 ± 2.2 mm) is shorter than in controls (14.9 ± 1.4 mm). The four basic LC neuron classes found in the normal human brain (large multipolar, large "bipolar," small multipolar, and small "bipolar" neurons; Chan-Palay and Asan: J. Comp. Neurol. this issue) are recognizable, but many cell somata are swollen and misshapen with foreshortened, thick, and less branched dendrites. LC neuron numbers are reduced (between −3.5% and −87.5%). Neuron loss is greatest in the rostral part, less in the middle, and least in the caudal part.

In PD cases, the rostrocaudal length (12.4 ± 1.5 mm) is shorter than in SDAT and controls. The neuronal morphology is more severely altered than in SDAT. The basic neuron classes are hardly distinguishable. Most cell bodies are swollen; they frequently contain Lewy bodies; and the dendrites are short and thin with absent or reduced arborizations. Neuron numbers are more reduced than in SDAT (between −26.4% and −94.4%). Alterations are as severe caudally as rostrally in P−D, and P+D/L-dopa nonresponsive cases. P+D cases are more severely affected rostrally. The presence of depression in SDAT and Parkinson's patients is accompanied by the greatest loss of LC neurons. On the basis of morphological alterations of the TH-immunoreactive neurons, and the degree and topographical distribution of neuron loss, a differentiation is possible between the LC in normal brain and that in SDAT and PD for diagnostic purposes.

Key words: tyrosine hydroxylase, immunocytochemistry, diagnosis

The locus coeruleus (LC), the most important source of norepinephrinergic (NE) input to almost every region of the brain (Foote et al., '83), has been shown to be affected in several neurologic and psychiatric disorders—namely, idiopathic and postencephalitic Parkinson's disease, Hallervorden-Spatz disease, Alzheimer's disease, paranoid schizophrenia, and a special form of endogenous depression (Van Dongen, '81). On the basis of results from animal experiments the LC has been postulated to influence sleep-wake cycles, the level of vigilance, and emotion (Chan-Palay and Asan, '89). Van Dongen ('81) has suggested that dysfunction of the nucleus and the central NE system may cause some of the mental impairment in the neurodegenerative diseases mentioned above.

Senile dementia of the Alzheimer type (SDAT) and Parkinson's disease (PD) are progressively disabling diseases, and their high incidence and prevalence rates in the aging society are of great significance. The outstanding feature of SDAT is a progressive dementia, which from certain clinical

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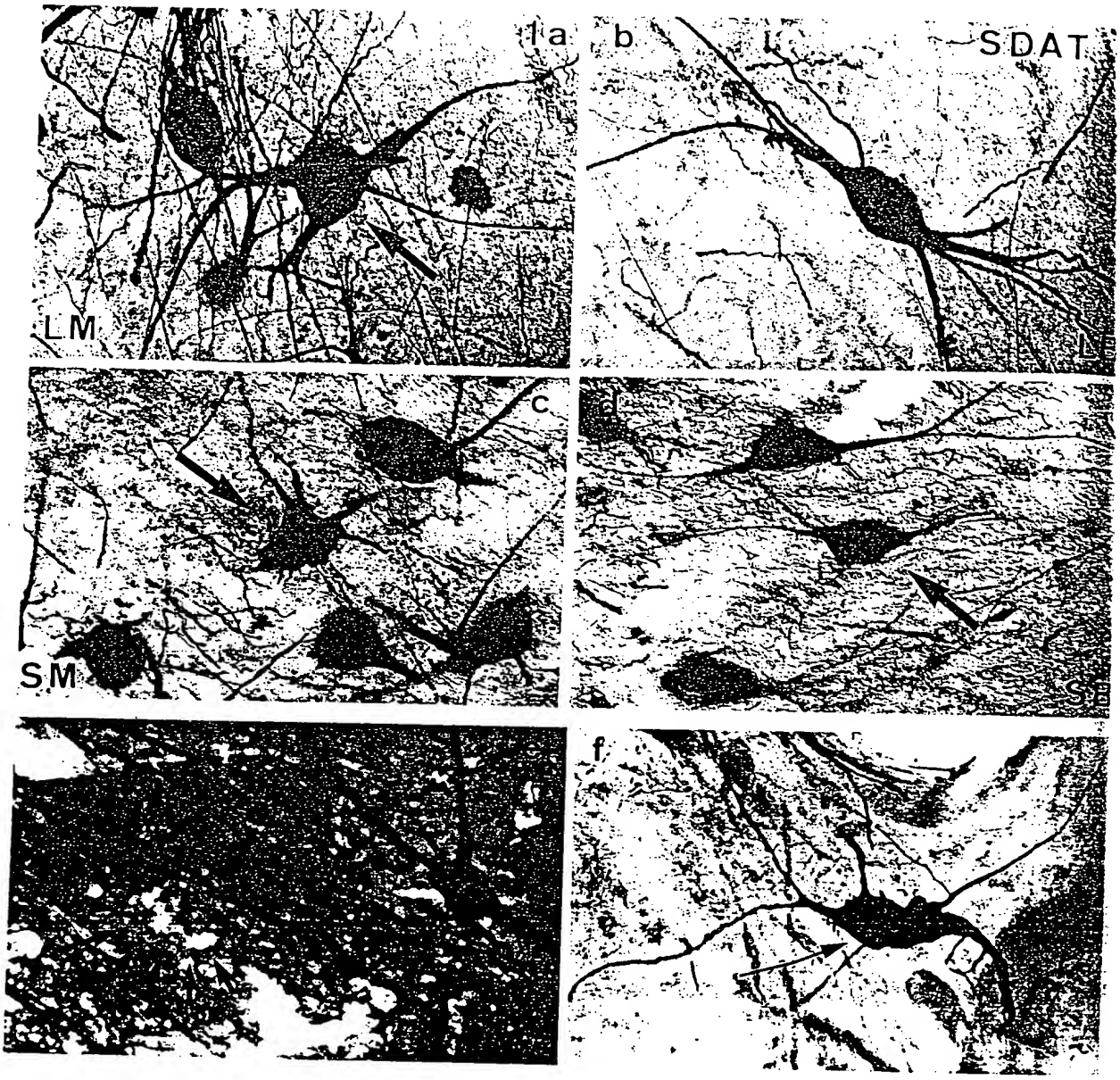


Fig. 1. Individual TH-immunoreactive neurons of the LC in SDAT. The four morphological classes typical for normal human LC (see Chan-Palay and Asan, '89, Figs. 1–3) are basically recognizable. The cell bodies of all neurons appear swollen, their outlines blurred. Dendrites are thick and occasionally foreshortened (a and b) and have few branches (c and d). a: Large multipolar (LM; arrow). b: Large "bipolar" (LB). c: Small multipolar (SM; arrow). d: Small "bipolar" (SB; arrow) neuron. e: Fluorescence microscopic picture of the LC in SDAT (thioflavin/TH immunocytochemistry double stain). Neuritic plaques (arrows) are seen among the dark TH-immunoreactive neurons and processes. f: Typical misshapen large cell of the "bipolar" type. The nucleus is bulging into the somatic outline (arrow). PAP method. ×450.

symptoms of amnesia, aphasia, and visuospatial disorder progresses to total disorientation, lack of motor coordination, incontinence, and aphasia. The main symptomatic features are generally attributed to a failure of function of certain cortical areas—mainly, the temporal and frontal lobes (Mann and Yates, '86). Neuropathologically, SDAT is characterized by severe cortical atrophy and cell loss as well as a high number of neurofibrillary tangles and neuritic plaques in the neocortex and hippocampus (Van Dongen, '81). In addition, several subcortical afferent projection systems are disturbed in the disease—namely, those based on acetylcholine, NE, and serotonin (Mann and Yates, '86, for review; Jellinger, '87; Chan-Palay, '88). The occurrence of extrapyramidal signs in SDAT (Mölsä et al., '84) suggests involvement of dopaminergic pathways in some cases (Jellinger, '87).

TABLE 1. Vital Data of Three Representative Age-Matched Control Cases, of Cases of Senile Dementia of the Alzheimer Type (SDAT), and of Cases of M. Parkinson Without (P – D) and With Dementia (P + D), of Cases of M. Parkinson Nonresponsive to L-Dopa Treatment (P + D/Dopa –), Including Counts of Neuritic Plaques (*) and Neurofibrillary Tangles (+) in Hippocampus CA1 and in Temporal and Parietal Cortex[1]

Case	Age	Sex	Postmort. delay	Clinical diagnosis	Cause of death	Ca1 hippoc.	Temp. cortex	Pariet. cortex	Sub. nigra
1	78	f	1.5	Control	Septic shock	0/0	0/0	0/0	—
2	79	m	6	Control	Pneumonia	0/0	0/0	0/0	—
3	83	f	10	Control	Myoc. infarct	0/+	0/0	0/0	—
4	71	f	3	SDAT	Pneumonia	***/+++	***/+++	**/++	—
5	72	m	5	SDAT	Broncho-pneumonia	***/++	**/++	*/+	—
6	74	f	9.5	SDAT	Pulmon. embolism	*/+++	*/++	0/+	—
7	77	f	4	SDAT	Pneumonia	***/+++	***/+++	**/++	—
8	78	m	16	SDAT	Pulmon. embolism	**/+++	***/+	*/+	—
9	78	f	5	SDAT	Broncho-pneumonia/depr.[2]	*/+++	**/+	0/+	—
10	84	f	14	SDAT	Pneumonia	**/+++	**/++	**/++	—
11	85	f	5	SDAT	Broncho-pneumonia	*/+++	**/++	*/+	—
12	76	f	7	(P – D)	Pulm. embolism	0/0	0/0	0/0	++
13	85	f	10.5	(P – D)	Pneumonia	0/0	0/0	0/0	++
14	83	f	14	(P + D)	Pulmon. embolism	0/0	0/0	0/0	++
15	85	m	21	(P + D)	Broncho-pneumonia	0/+	0/0	0/0	++
16	90	m	6	(p + D)	Cardiac insuff.	*/0	0/0	0/0	++
17	79	m	5.5	(P + D/dopa –)	Broncho-Pneumonia/depr.[2]	0/+	0/0	0/0	++
18	82	m	3.5	(P + D/dopa –)	Pneumonia/depr.[2]	*/0	0/0	0/0	++

[1]Counts made at 250× magnification in a total of 20 visual fields, expressed in number per sq. mm. 0 – minimum plaques or tangles (0–1); *, + – few plaques or tangles (2–10); **, ++ – moderate plaques or tangles (10–20); ***, +++ – numerous plaques or tangles (>20). Substantia nigra shows gliosis, cell loss, neuron pigmentation and Lewy bodies: ++; or no changes: —.
[2]depr – depression.

Investigations of the functional role of the LC-NE system in SDAT have previously focused on the study of LC cellular neuropathology and measurements of NE content in the various cortical projection areas of the LC. Quantitative investigations using the neuromelanin pigment as a marker for NE neurons have demonstrated a reduction of neuron numbers in the LC in most cases of SDAT (Tomlinson et al., '81; Mann et al., '82, '83, '84; Bondareff et al., '82) with a high incidence of neuropathologic markers such as neurofibrillary tangles and neuritic plaques and, occasionally, Lewy bodies in the remaining neurons. Recent studies using catecholamine biosynthetic enzymes have also demonstrated a loss of LC-NE neurons in SDAT (Iversen et al., '83; Ingram et al., '87a,b), though with differing total neuron numbers in control and SDAT cases (approx. 9,500 and 4,100 neurons for controls and SDAT cases respectively in Iversen et al., '83, and 27,600 and 7,700 in Ingram et al., '87a). This cell loss from the LC was reported to be topographically distributed (Lockhart et al., '84; Marcyniuk et al., '86; Ingram et al., '87a,b). NE level, dopamine-β-hydroxylase (DBH) activity, and the levels of several other NE markers have been shown to be decreased in LC projection areas both in antemortem biopsies (Palmer et al., '87) and in postmortem brain tissue (Mann et al., '82; Iversen et al., '83; Arai et al., '84; Lockhart et al., '84), indicating a deficiency of the NE transmission in SDAT. Correlation between cortical plaque formation (Marcyniuk et al., '86), cortical NE levels (Lockhart et al., '84), and LC neuron loss in the anterior and central regions of the LC known to project to these areas in animals (Mason and Fibiger, '79; Loughlin et al., '82) have been reported. Also, the severity of cortical plaque incidence has been correlated with the degree of reduction of LC neuron number (Tomlinson et al., '81). Though suggested by this author, no direct correlation between the severity of dementia and the extent of LC damage has been demonstrated. One recent study, however, has shown a positive correlation of the occurrence of depression in SDAT with the decrease in LC neuron number (Ross et al., '87).

Lesions of brainstem nuclei including the LC with neuropathologic changes such as Lewy bodies and neurofibrillary tangles in PD have been described many years ago (Greenfield and Bosanquet, '53), and the Parkinsonian state and LC lesions similar to those found in the disease are caused by the administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the macaque monkey (Mitchell et al., '85). Even though PD is principally a disorder of locomotion, it is now generally accepted that in a number of patients progressive mental impairment occurs in the course of the disease. Some authors have reported dementia in more than 50% of cases of PD (Hakim and Mathieson, '79; Wettstein et al., '86). According to the responsiveness to L-dopa treatment, Lieberman et al. ('79) have postulated that two separate disorders can be distinguished in PD: an exclusively motor disorder occurring in a younger population with a longer and more benign course and a better response to L-dopa; and another, a motor followed by a cognitive disorder occurring in an older population with a more fulminant course and a poorer response to L-dopa. There exists a controversy over the distinction of "cortical" dementia found in SDAT and "subcortical" dementia present in PD patients. Several authors have claimed that in neuropsychological tests the dementia of SDAT, characterized mainly by aphasia, amnesia, agnosia, and apraxia, can be distinguished from that found in PD patients, in whom the dementia is characterized by slowness of mental processing, forgetfulness, impaired cognition, apathy, and depression (Cummings and Benson, '84; Huber and Paulson, '85; Huber et al., '86), while others have found no psychopathological difference between demented PD and SDAT patients (Mayeux et al., '83; Wettstein et al., '86). Whitehouse ('86), reviewing clinical, neuropathological, and neurochemical studies, finds little support for classifying dementias into "cortical" and "subcortical" types. Some investigators have suggested that the dementia in PD displays a pattern of impairment typical for a lesion of the frontal lobe (Caltagirone et al., '85), and a laterality of the disease has been suggested on the basis of the finding that patients with greater disease involvement on the left side of the body showed greater neuropsychological impairment than those more affected on the right side (Direnfeld et al., '84).

The question of whether the incidence of cortical plaques and tangles is correlated to the severity of dementia in PD is still also somewhat controversial. Early reports have shown a more frequent occurrence of neuritic plaques and neurofibrillary tangles in demented PD patients than in nondemented (Boller et al., '80; Boller, '85), thus suggesting coincidental SDAT in these patients. Other authors could not demonstrate a positive correlation between neurofibrillary

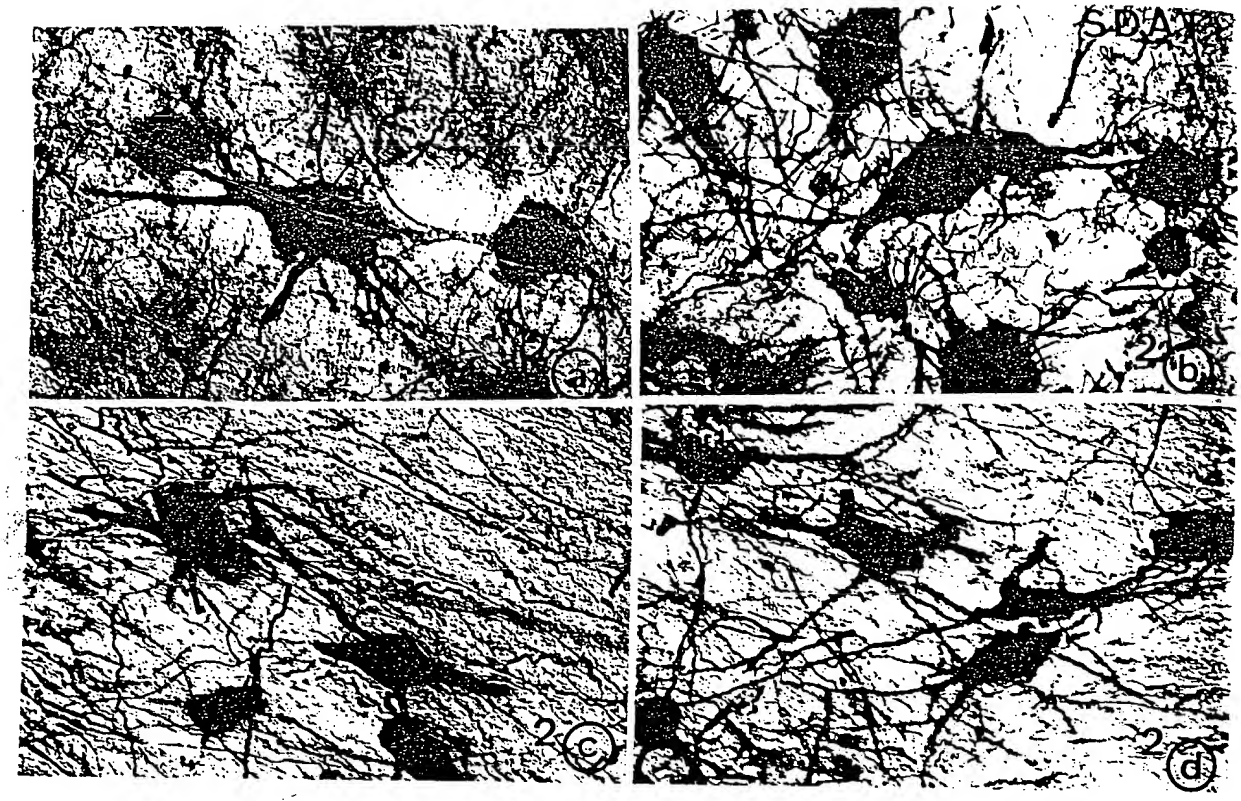


Fig. 2. Groups of TH-immunoreactive neurons in the LC in SDAT. Typical misshapen cell bodies of large (a and b) and small cells (c and d) with thick and short dendrites. PAP method. ×450.

tangle and neuritic plaque formation and dementia but reported a severe cell loss in the LC more frequently in demented PD patients than in those without symptoms of dementia (Mann and Yates, '83; Gaspar and Gray, '84). A correlation between the coeruleocortical NE system and dementia has also been suggested on the basis of modifications in the number of adrenergic receptors in demented PD patients (Cash et al., '84).

In view of the findings concerning the LC in SDAT and PD briefly reviewed above, it is desirable to have a more precise knowledge of the LC alterations on a cellular, quantitative, and qualitative basis, employing chemically specific methods. Our goal in the present study is to investigate in detail the morphological alterations of chemically identified NE-synthesizing LC neurons and the nucleus as a whole in patients suffering from SDAT and in demented and nondemented patients with PD by using computer-assisted morphological quantitations and analyses and to compare these results with the normal morphology of the nucleus and the LC in a depressed patient (Chan-Palay and Asan, '89). We hope to provide clear information from postmortem studies of the NE neurotransmitter system in these dementing disorders as a step towards a systematic understanding of the transmitter deficiencies in these diseases. We expect also to provide the means for a differentiation between these various dementias based upon differences in the changes wrought in the locus coeruleus NE system.

MATERIALS AND METHODS

The Zürich study on dementia is an interdisciplinary pr gram to follow longitudinally a group of demented patien and their age-matched nondemented cohorts permanent residing in the chronic care hospitals for the elderly. Th patients are selected on the basis of their clinical symptor atology and neurological findings, regardless of exact a and sex. However, the majority of our cases are between th ages of 70 and 100 and the proportion of females is highe than males, reflecting the general demographic trends i society at large. The cases are placed in the following categ ries: 1) senile dementia of the Alzheimer type (SDAT); Parkinson's disease (L-dopa responsive) without dement (P – D); 3) Parkinson's disease (L-dopa responsive) with de mentia (P + D); 4) Parkinson's disease or a Parkinsonismu (L-dopa *non*responsive) with dementia (P + D/L-dopa nor responsive); 5) age-matched controls; 6) young contro (ages 40–60). The patients are clinically followed for th total period of hospitalization (from 1½–5 years) to exitus. I this period a psychometric test battery consisting of laterai ization index, activities of daily living, socialization scal minimental (Zürich variant) status, Hamilton depressio scale, involuntary movements and extrapyramidal move ments scale, and memory tests are administered every months in addition to complete neurological examinations After exitus, all cases go to autopsy and a special transpor

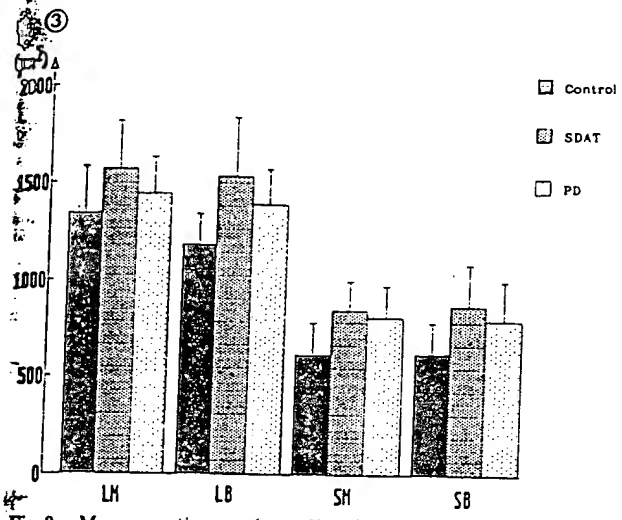


Fig. 3. Mean somatic areas (n > 10) and standard deviations of TH-immunoreactive LC neurons of different morphological classes (LM: large multipolar; LB: large "bipolar"; SM: small multipolar; SB: small "bipolar" neurons) of a paradigm age-matched control case (#3), an SDAT (#7), and a PD case (#18).

stem is arranged in order to attain a shorter postmortem lay before autopsy. Cases with exitus over the weekends holidays are dropped from the study at this stage.

In the present study normal controls included the brains 12 patients, four male and eight female, ranging in age om 43 to 89 years, with no clinical history of neurological psychiatric disease as confirmed by postmortem gross d microscopic neuropathological examination. Vital data d selection criteria for all control cases were described in tail in the previous paper (Chan-Palay and Asan, '89). ta assembled in three paradigm control cases (Table 1) in e age group of the patients in the Alzheimer and Parkin- groups served as control values for quantitative analy- Appropriate levels of normal cognitive function in tients were shown by results of between 22 and 26 from a ssible 30 points in the last available minimental status ts. One case, #18, had an 18 year history of chronic pression without dementia, and a minimental status of m 26.5 and 29 in multiple tests over 2 years (Chan-Palay d Asan, '89).

In the group of senile dementia of the Alzheimer type DAT), the brains of eight patients that had been clinically gnosed were studied, two male and six female subjects h ages ranging from 71 to 85 years. Cases of dementia due other neurological disorders, such as ischemia, multiple arcts, Pick's disease, etc., were excluded. Postmortem de- s ranged from 3 to 16 hours, with postmortem delays of 5 rs and less in five of the cases (Table 1). Counts of neu- c plaques and neurofibrillary tangles were made on Bod- -silver-stained preparations at ×250 magnification in a al of 20 separate visual fields in the hippocampus CA_1 l the temporal and parietal cortices. Mean values were en for these fields and expressed in number per square limeter of tissue examined for each separate brain ion. For all cases in this group, the counts yielded moder- to high indices of neurofibrillary plaques and tangles in examined areas, which is indicative of SDAT (Table 1). iously impaired mental function in these patients was icated by a score of 0 to 5 points in the last available minimental status tests. SDAT case #9 had a concurrent depression.

TABLE 2. Mean Somatic Areas (n > 10) and Standard Deviations of TH-Immunoreactive LC Neurons of Different Morphological Classes of a Paradigm Control Case (#3), an SDAT (#7), and a PD Case (#18)

Case (no.)	Age	Sex	Mean somatic area (μm^2)[1] LM	LB	SM	SB
Control (3)	83	f	1,340 ± 233	1,177 ± 155	614 ± 164	622 ± 155
SDAT (7)	77	f	1,563 ± 242	1,527 ± 296	843 ± 147	865 ± 212
PD (18)	82	m	1,435 ± 183	1,383 ± 177	803 ± 167	795 ± 197

[1]LM: large multipolar; LB: large "bipolar"; SM: small multipolar; SB: small "bipolar" neurons.

TABLE 3. Quantitative Data for Morphology of Individual Cells of Different Classes for an Age-Matched Control (Cont), a Case of SDAT, and a Case of Parkinson's Disease With Dementia (P+D), and the % Change

Cell[1]	Diagnosis (case no.)	Age	Sex	Soma area (μm^2)	% change from control	Total arbor length (μm)	% change from control
LM	Cont (3)	83	f	1,233	—	1,919	—
	SDAT (9)	78	f	1,691	+37	481	−75
	P+D (14)	83	f	1,214	−1.5	352	−82
LB	Cont (3)	83	f	1,345	—	2,177	—
	SDAT (9)	78	f	2,158	+60	808	−63
	P+D (14)	83	f	1,454	+8	531	−76
SM	Cont (3)	83	f	558	—	710	—
	SDAT (9)	78	f	891	+60	237	−67
	P+D (14)	83	f	635	+14	218	−69
SB	Cont (3)	83	f	516	—	1,034	—
	SDAT (9)	78	f	711	+38	316	−69
	P+D (14)	83	f	740	+43	292	−72

[1]LM: large multipolar; LB: large "bipolar"; SM: small multipolar; SB, small "bipolar" cells.

In the study of cases with Parkinson's disease seven diagnosed patients were included, ranging in age from 76 to 90 years, three male and four female cases. Clinically, two of the patients had PD responsive to L-dopa treatment without symptoms of dementia; five patients had histories of rapidly progressive dementia, with onset 2–3 years before death. Of these five demented patients, three were responsive to treatment with L-dopa; two were atypically depressed and their Parkinsonian symptoms did not respond to L-dopa treatment. The postmortem delays ranged from 3.5 to 21 hours and were less than 7 hours in four of the cases (Table 1). The clinical diagnosis of Parkinson's disease was confirmed at autopsy by both gross and microscopic neuropathological examination. The substantia nigra showed considerable cell loss, loss of pigmentation, numerous Lewy bodies, and gliosis pathognomic of Parkinson's disease in every case. Counts of neurofibrillary tangles and neuritic plaques were performed as described for the SDAT cases and yielded indices slightly higher for the demented Parkinson's patients than in the age-matched control and nondemented Parkinson's cases (Table 1). The last available minimental status test scores were 22–26 for the nondemented Parkinson's disease group and 11–17 for the demented patients.

Fixation and immunocytochemistry

The protocols used for fixation of the studied brainstems and immunocytochemistry were described in detail in the preceding paper (Chan-Palay and Asan, '89). Briefly, at autopsy the brains were perfused with 4% paraformalde-

LM
LB
SM
SB
CONTROL
SDAT
PARK
a
b
c
d
e
f
g
h
i
j
k
l

de (PFA); the brainstems were dissected and postfixed in FA and Bouin's fixative. From the part of the brainstem ntaining the LC 70 μm-thick serial vibratome sections ere cut in the coronal plane and collected in series of every fth section. One series was Nissl stained to confirm details f nuclear boundaries and serve as morphological reference, nd immunocytochemical demonstration of tyrosine hydroxylase (TH) was performed on the other series. In the ajority of cases, two adjacent series of sections were incubated with antiserum against TH (Eugene Tech, NJ) at a dilution of 1:1,200 and the reaction product in one series was visualized with the peroxidase-antiperoxidase (PAP) method with 3,3'-diaminobenzidine (DAB; Sigma Chemical Co.) as the chromogen. In the second series it was visualized ith the immunogold-silver-staining (IGSS) method. Antibody specifications and technical details for reactions and controls have been described (Chan-Palay and Asan, '89). After comparison of the two visualization methods had howed that the results were equivalent, in the remainder of ases only one series of sections was incubated with antibodies against TH and visualized with the PAP method. Those euronal somata and processes in the LC that showed a specific reaction with antibodies against TH are referred to as TH immunoreactive in this paper and are presumed to be atecholaminergic and the terms are used interchangeably. Though the antiserum used in this study is of proven specificity, we are aware of the possibility that the antibody may ecognize or provide positive reactions with similar or elated molecules or as yet unknown molecules.

Double label with thioflavin for neuritic plaques

Every fifth section of the TH series of brainstem sections om the cases of SDAT was counterstained with 1% thioavin (Sigma) for 30 minutes, differentiated in 70% alcohol, nd dehydrated in an ascending alcohol series prior to covrslipping with Eukitt (Bern, Switzerland). These preparaions were examined with fluorescence in addition to transuitted light microscopy to determine coexistence of H-immunoreactive elements in neuritic plaques identified y the amyloid stain with thioflavin (Chan-Palay et al., '85, 6).

Computer-assisted quantitative morphological analyses

The computer system and the recording procedures used ve been described in detail in the preceding paper (Chanalay and Asan, '89). Briefly, the immunocytochemically ained serial brainstem sections were reassembled in the rrect anatomical order; the LC was outlined in the coronal ane (Olszewski and Baxter, '54), its rostral and caudal borrs were determined, and its rostrocaudal length on both sides of the brainstem was calculated. For the computer-assisted measurements of morphological parameters of TH-immunoreactive LC neurons and for the mapping and counting of neurons and the three-dimensional reconstruction procedure for the analysis of neuron distribution in the LC an IBM-AT-mouse-based user-interactive image analysis system with the Cellmate program (Bioquant, TN) was used. Outlines of individual cell somata and dendritic arbors were recorded for calculations of somatic areas and dendritic arbor length. Plots of these recordings served to illustrate alterations in individual neuron morphology. To ensure comparability all quantitative measurements of neuronal parameters were carried out on immunoreactive neurons stained with the PAP method. Cell counts were performed on all reassembled sections of one TH-immunoreacted series of sections by cursor-marking the location of whole cell bodies with different symbols signifying different morphological classes of cells in all focal planes throughout the entire extent of the LCs of both sides of the brainstem. Neuron numbers on partially damaged sections were approximated either from the numbers counted on immediately adjacent TH-immunoreacted sections or from those counted in the contralateral LC of the same section and the ipsilateral LCs of the preceding and following sections of the same series. Total neuron numbers were calculated by the computer for the entire LCs and, by their differing recording symbols, differentiated according to the four neuron classes: Large multipolar (LM), large "bipolar" (LB), small multipolar (SM), and small "bipolar" (SB) neurons. The recordings of the reassembled sections were then aligned to match as closely as possible the situation in the intact brains, and an image of the three-dimensional distribution of the neurons was created by the computer. To facilitate comparison of three-dimensional distributions of neurons in the LC in brains of normal adults and of the neurologically diseased, the four LC-typical neuron classes were assigned different colours, and colour photographs of these reconstructions were made. Additionally, for the three-dimensional reconstruction of the solid shapes of the LCs and the fourth ventricle the perimeters of the catecholamine cell groups of the LCs on both sides of the brainstem were drawn on all aligned sections. Polygons were calculated from the outlined perimeters and an image of the structures in which they appeared as smoothly shaped solid bodies was created, thus facilitating the detection of differences in length and shape of the loci of both sides and of possible laterality. Black-and-white photomicrographs were made with a Zeiss photomicroscope fitted with Nomarski optics. Colour photographs of reconstructions were made directly from the monitor.

RESULTS

The morphological features found in the normal age-matched human LC described previously (Chan-Palay and Asan, '89) will be used as a basis for discussing disease-related alterations. The classification of TH-immunoreactive LC neurons into the four groups, large multipolar (LM), large "bipolar" (LB), small multipolar (SM), and small "bipolar" (SB), defined in the normal LC, was used for typifying the LC neurons in SDAT and Parkinson's disease. However, in the SDAT and Parkinson cases, alterations in neuron morphology and size made the basic features often difficult to recognize. The presence of neuritic plaques, torpedo formation, and Lewy bodies complicated the process of

ig. 4. Plots from the computer recordings for quantitative morphoical analysis of individual neurons of the four typical LC-NE neurons control (a–d), SDAT (e–h), and PD (i–l) brains by means of the emate program. The first column of neurons consists of the large ltipolar (LM) class cells; the second column of the large "bipolar") class cells; in the third column the small multipolar (SM) class cells in the fourth column the small "bipolar" (SB) class cells are shown. characteristic changes observed in the diseases are easily seen. Mispen cell bodies and short dendrites with few branches in SDAT, mp cell bodies and even shorter dendrites with fewer branches in

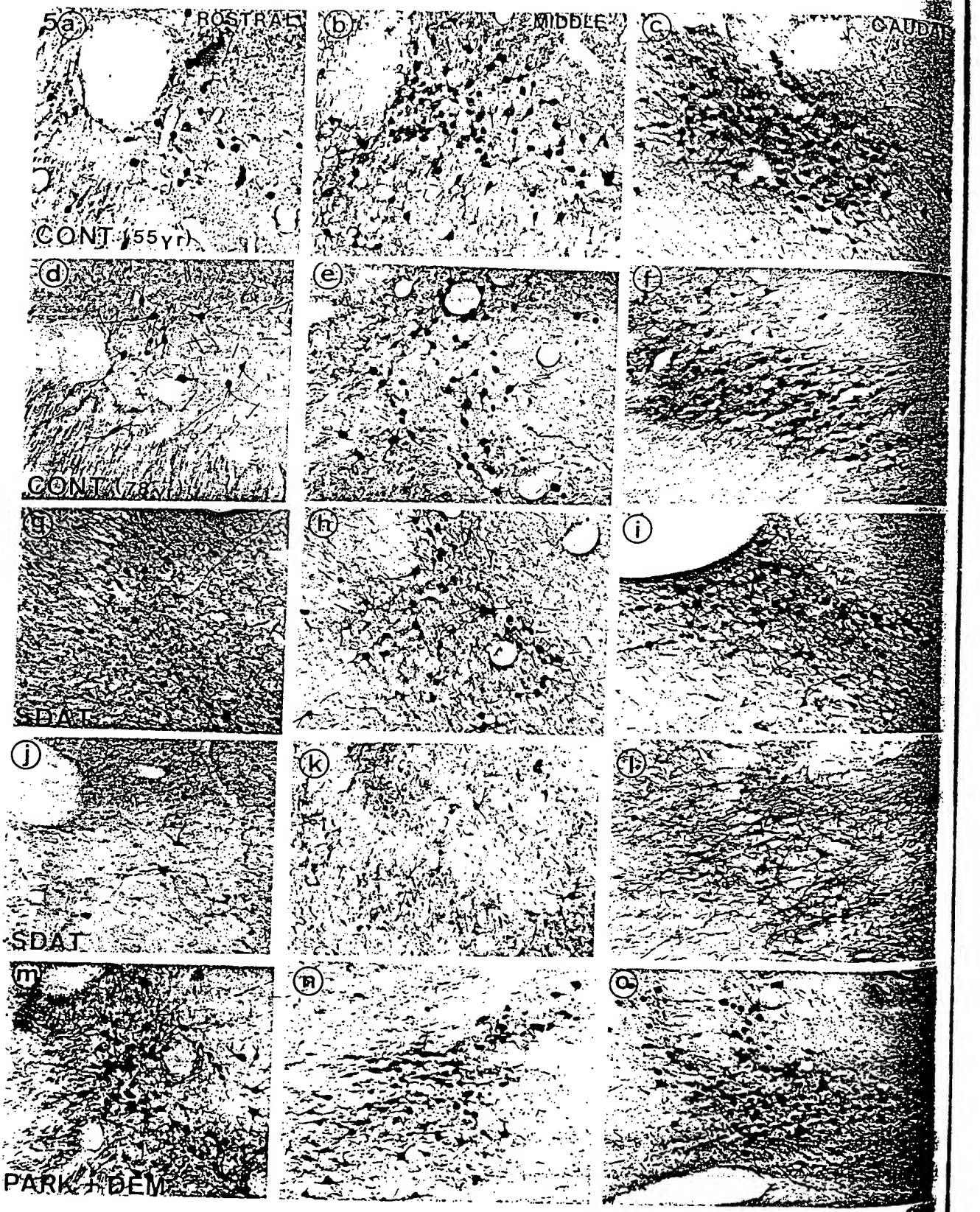

5a
ROSTRAL
b
MIDDLE
c
CAUDAL
CONT (55 yr)
d
e
f
CONT (78 yr)
g
h
i
SDAT
j
k
l
SDAT
m
n
o
PARK + DEM

Figure 5

differentiation further. Recognition of the three subdivisions of the LC found in normal human adult brain was straightforward since their boundaries were defined by anatomical landmarks which remain unchanged in the disease. In controls, the rostral LC part extending from the first appearance of LC neurons to the decussation of the trochlear nerve, the middle part extending to the point where the inferior part of the mesencephalic trigeminal tract is no longer enveloped by the main body of the LC, and the caudal part extending to the caudal group of LC neurons in the main body of the LC were easily defined in all cases included in this study. Comparison of PAP and IGSS methods on adjacent series of sections of the same brainstem confirmed the equivalence of the two methods for demonstration of TH immunoreactivity. Since the IGSS method yielded higher contrast between specific reaction product and background and the PAP method allowed a better study of neuronal morphology, both were used for neuron counts and the PAP method was used for the details of neurons (Chan-Palay and Asan, '89).

Cases of SDAT

The degree of alterations with respect to both neuron morphology and cell numbers in the different cases of SDAT proved to be extremely variable, probably reflecting the extreme variability of the clinical disease in individual patients. Despite the variability of the changes, some features and changes were common to the majority of cases. An important aspect is that a low minimental test result or a minimental status of 0 in the patient correlated with severe destruction in catecholamine neurons in the LC.

LC neuron morphology. Six of the eight cases displayed alterations of cell morphology throughout the nucleus. In the remaining two cases neuronal change was restricted to the rostral part (cases #10 and 11). The basic classification into the cell groups LM, LB, SM, and SB was still possible (Fig. 1a–d). Even in the comparatively normal-appearing neurons, however, the somatic outlines were blurred and the dendrites were thicker and shorter than normal. Especially in the groups of larger cells, many neurons had swollen and misshapen cell bodies in which the nuclei were displaced to the periphery, causing bulges in the somatic outline (Figs. 1f, 2a,b). In cells of all classes, dendritic arbors were less branched, appeared foreshortened and gnarled, and often ended with large terminal diameters near their stem from the cell body (Fig. 2a–d). Immunoreactivity of the neurons varied within and between individuals. The majority of neurons displayed somewhat more intense immunoreactions than controls, but some neurons were weakly reactive. Fluorescence microscopy of the thioflavin S-stained sections showed that small neuritic plaques could be observed occasionally between the LC neurons (Fig. 1e).

Fig. 5. Low-magnification photomicrographs of TH-immunoreactive sections, showing the LC of one side at a rostral, middle, and caudal levels in a younger (55 year; a–c) and an older (78 year; d–f) control, an SDAT case with comparatively little cell loss (g–i), an SDAT case with high cell loss(j–l), and a PD case with dementia, L-dopa nonresponsive (m–o). (For a full description of the younger control case see text Chan-Palay and Asan, '89; please use for comparison with the other four cases). Note that the TH-immunoreactive neuronal morphology, the topographic localization of the neuronal changes, and the total numbers of remaining neurons in each region allow a clear diagnostic differentiation between the LC of controls, SDAT, and PD. PAP method. ×90.

Quantitative analyses

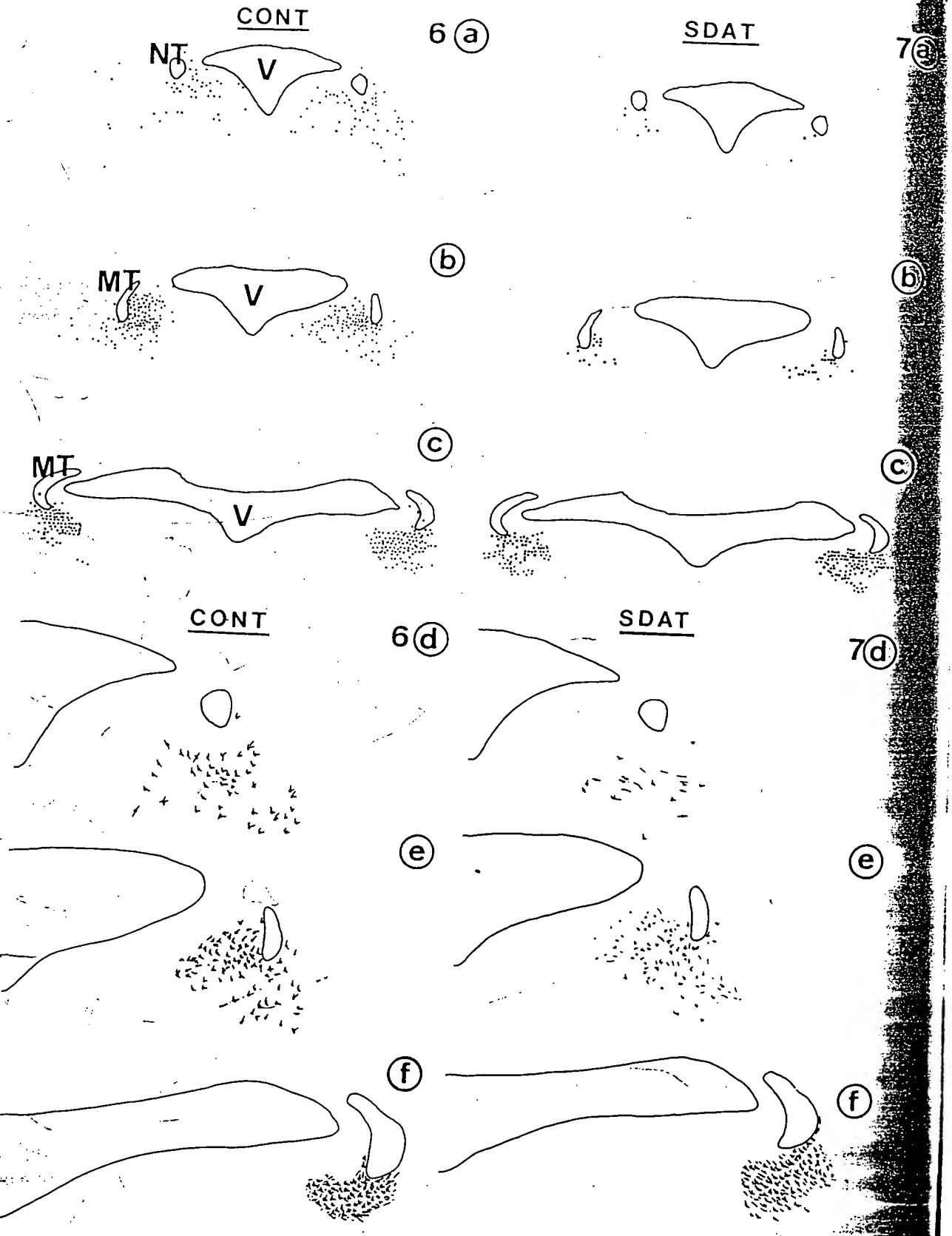
Somatic areas. Computer-assisted measurements of the somatic areas of TH-immunoreactive neurons of the different cell classes were carried out on selected sections of a paradigm case of SDAT (case #7). More than ten somata of neurons in each class were measured. Figure 3 and Table 2 show the mean values ± standard deviation for the somatic areas of this case together with those of an age-matched control (case #3) and a case of PD (case #18).

Quantification confirmed the observation that the somatic areas in all classes were considerably larger than in controls (Table 2; significant on a 99% level, U-test). Measurements of cells of other cases confirmed these somatic size ranges.

Arbor parameters. Cells from each of the four classes from controls (Fig. 4a–d) and SDAT cases (Fig. 4e–h) were analysed for somatic areas and dendritic arbor length. Care was taken to ensure that the quality and intensity of immunoreactivity were comparable. Table 3 shows a typical result obtained for one set of cells from case #3 (control) and case #9 (SDAT). While the somatic areas of the neurons recorded were all larger than those of controls (between +37% and +60%), the total arbor length was extremely reduced in cells of all classes (between −63% and −75%). These quantitations confirmed the observation that the dendritic arborization in LC neurons of SDAT cases was less extensive than in controls. Computer drawings of the SDAT neurons from all classes from which the quantitative measurements were calculated illustrated the typical alterations seen in this disease (compare Fig. 4a–d with 4e–h).

Nuclear morphology. The typical regional distribution, with scattered cells rostrally, more densely packed cells in the middle, and tightly clustered cells caudally, of the LC in age-matched controls (Fig. 5d–f; compare Chan-Palay and Asan, '89), was still recognizable in SDAT cases (Fig. 5g–i). In cases with extremely low cell numbers, however, the morphological characteristics of the different parts could not be distinguished any more because of the reduced cell densities (Fig. 5j–l). Alterations were generally most notable in the rostral, less so in the middle, and least in the caudal part of the LC. Plots of the computer recordings of sections from the three LC parts showed that compared to the controls (Fig. 6a–c) both the area of the LC and the cell density were extremely reduced in the rostral and middle parts of the LC in SDAT (Fig. 7a–c) while the caudal part was relatively spared. The subjective impression that the alteration of the LC in SDAT compared to the age-matched control was due to changes in neuron morphology and reduced dendritic arborization was documented by low-magnification camera lucida drawings of the LC (control: Fig. 6d–f; SDAT: Fig. 7d–f). More extraneuronal pigment was observed in the diseased loci, especially in the rostral part. In this region of the nucleus, the number of pigmented neurons that showed weak TH immunoreactivity was higher in SDAT cases than in controls. Few nonpigmented neurons displayed TH immunoreactivity in SDAT and the dendritic network surrounding the neurons was less extensive than in controls.

Axonal terminal fields. The density of TH-immunoreactive axons and axon terminals, which could be identified only with difficulty and in fragments, was less than in controls throughout the LCs, especially where cell loss was high.



Figures 6 and 7

TABLE 4. Average Rostrocaudal LC Length, Standard Deviations (SD), and Mean Differences Between Left and Right LCs in Control (n = 10), SDAT (n = 8), and PD (n = 7) Cases

	mm		%	
gnosis	Length	SD	SD/length	Mean side-diff.
ntrol	14.9	1.4	9.1	2.9
AT	13	2.2	16.7	3.7
	12.4	1.5	12.3	0.7

Quantitative analyses

Total length of the LC. The rostrocaudal length of the clei was determined in all eight SDAT cases. The average ngth was reduced from that in controls (13 and 14.9 mm spectively; Table 4) and the standard deviation of the val- s was higher (2.2 and 1.4 mm respectively), thus also indi- ating different degrees of alteration. Length differences of e LCs of left and right sides ranged from 0 to 15.2%, aching higher values than in controls (mean side differ- ce in SDAT 3.7%, in controls 2.9%).

Neuron numbers. In six of the eight cases (cases #4, 6– and 11), cell counts were done for the LCs of both sides of brainstem and compared to average numbers of the ree age-matched control cases (Table 5). Differentiation the cells according to their classes was carried out in three these cases (cases #4, 8, and 9; Table 6). The cell loss (Ta- 5) compared to age-matched controls ranged from mini- l (case #11; 3.5%) to extreme (case #7; 87.5%), with dif- ences in cell counts between left and right sides ranging m 1.8% to 17.1% compared to 1.0–6.6% in the age- tched controls. In four of the six cases counted, the later- y differences were higher than in the age-matched con- ls. A distinction between the results from the two sexes uld not be extracted from these data because of the fact t only one of the cases quantitated was male. In all cases, l loss was extreme in the rostral part (more than 67% in of the six cases); in the middle part the cell loss ranged m none (case #11; +6.9% of controls) to extremely high se #7; −97.6%). The caudal part was generally least cted by cell loss (Table 5). In case #11, the cell number he caudal part was considerably higher than in controls 12.7%).

The relative contribution of the different morphological sses of LC neurons varied greatly between the SDAT es. In the rostral LC in cases #8 and 9 the contribution of e cells was about the same as in controls; their relative nber was higher rostrally in case #4 and in the middle and caudally in all cases. In cases #8 and 9 SM cells were more frequent than SB cells rostrally, gradually decreasing caudally, and became fewer than SB neurons in the caudal part. SM cells were more frequent than SB neurons throughout the LC in case #4 (Table 6).

Cases of Parkinson's disease

The LC from brains of patients in this group displayed extremely varying degrees of alterations. The results were analysed for the overall group as well as for the subgroups of P−D, P+D, and P+D/L-dopa nonresponsive.

Neuronal morphology. Typical for the majority of cases (cases #12–14, 17, and 18) was the especially dramatic cell destruction, rendering the cells of the different morphological classes barely recognizable. Cell bodies were rounded and misshapen with blurred somatic outlines. Dendrites were thin, short, and often gnarled, and some neurons seemed to be devoid of processes (Fig. 8a–d). Remnants of destroyed cells and masses of extraneuronal pigment were found in the neuropil surrounding the remaining LC neurons, which was almost devoid of the network of TH-immunoreactive fibers found in controls (Fig. 9a–c). Lewy bodies were found in many neurons (Fig. 9d). Occasionally, very well-preserved neurons could be found in completely destroyed neuropil. Immunoreaction product was extremely unevenly distributed within the neurons, and both intensely and very weakly reactive neurons could be found in the same area of the LC. The extreme neuronal alterations observed in the majority of cases gave the impression that the structural integrity of the neurons was destroyed. Such alterations were present throughout the entire loci in both P−D cases (#12 and 13), in both P+D/L-dopa nonresponsive cases (#17 and 18), and in one of the P+D cases (#14). In the P+D cases #15 and 16 extraneuronal pigment and signs of cell degeneration were observed mainly in the rostral part.

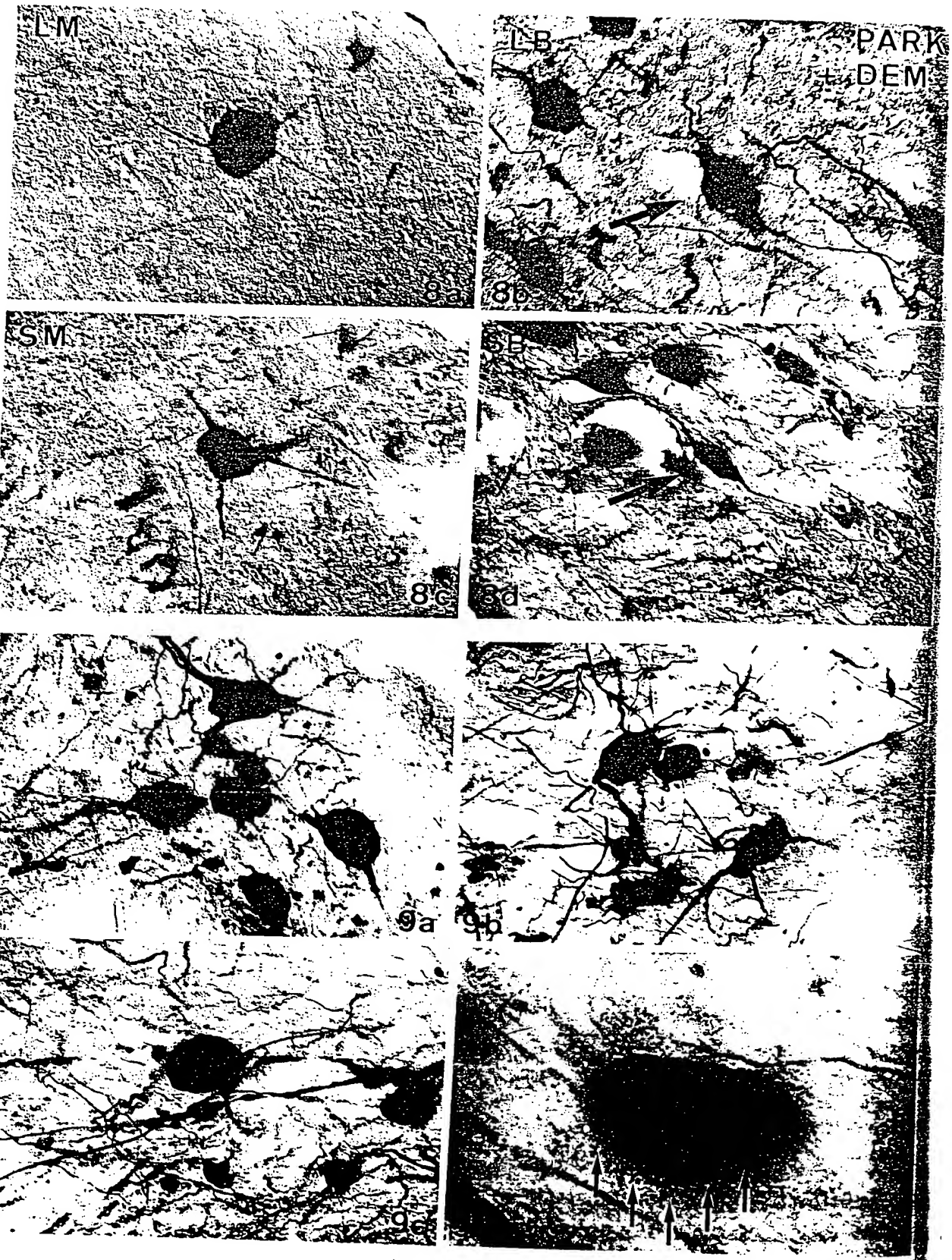
Quantitative analyses

Somatic areas. Computer-assisted measurements of somatic areas of cells that could be categorized into the four classes were carried out in case #18 (Fig. 3 and Table 2). More than ten somata were measured in each morphological class. The increase in somatic area compared to the control was notable, but not quite as high as in Alzheimer cases (not significant for LM class cells, significant on a 99% level for the other three classes; U-test). Measurements of cells of other cases confirmed somata to be in a similar size range.

Arbor parameters. Paradigm cells of each of the four classes typical for Parkinson cases of all three groups were analysed for somatic areas and dendritic arbor length, as described. Care was taken to choose comparably immunoreacted control and Parkinson cases (cases #3 and 14 respectively; Table 3). While the somata of the chosen cells were about the same as or larger than those of controls (−1.5% to +43%), the arbor length was even more reduced in the Parkinson than in the SDAT cells (between −69% and −82% of the control values), confirming the qualitative observations. The qualitative and quantitative observations on characteristic LC neuronal morphology were illustrated in the computer drawings of neurons of the different morphological classes (Fig. 4i–l).

Nuclear morphology. Excepting cases #15 and 16, the loci in all three groups of Parkinson cases were more severely altered than in SDAT. Masses of extraneuronal pigment and cell remnants were found throughout the

gs. 6, 7. The distribution of TH-immunoreactive LC neurons in al (a,d), middle (b,e), and caudal (c,f) parts of an age-matched con- nse (Fig. 6a–f) and an SDAT case (Fig. 7a–f). The plots from Fig- 6a–c and 7a–c have been made from the quantitative computer dings of TH-immunoreactive neurons. The remaining figures from res 6d–f and 7d–f were drawn by camera lucida from the same sec- to show neuronal form and distribution. Outlines of morphological ence structures are marked in Figure 6a–c: V signifies the fourth ricle; NT, the trochlear nerve; and MT, the mesencephalic trigemi- ract. Note that the cell densities are reduced in the SDAT case com- d to the control, especially in the rostral and middle parts. The cau- art is relatively spared from cell loss.



Figures 8 and 9

TABLE 5. Comparative Numbers (1 × 10^3) of the TH-Immunoreactive Neurons in the Rostral (Ro), Middle (M), and Caudal (C) Regions of the Loci Coerulei of Both Sides of the Brainstem of Controls (Ctr: Mean Values of Cases: 4, 6, and 7 With Standard Deviation, std), 6 Cases of SDAT, 7 Cases of PD—Two Without Dementia (P – D), Three With Dementia (P + D), and Two With Dementia, L-Dopa Nonresponsive (P + D/Dopa –) — and the % Loss of Neurons in the Diseased as Compared to the Mean Control Values

Case no.	Age	Sex	Ro	% neuron loss	M	% neuron loss	C	% neuron loss	Tot	% neuron loss
Ctr (std)			7.20 ± 0.63		26.00 ± 1.70		11.85 ± 1.48		45.03 ± 2.96	
SDAT (4)	71	f	0.33	−95.4	5.38	−79.3	3.97	−66.5	9.67	−78.5
SDAT (6)	74	f	1.43	−80.1	8.88	−65.8	8.52	−28.1	18.83	−58.2
SDAT (7)	77	f	0.22	−96.9	0.63	−97.6	4.8	−59.5	5.65	−87.5
SDAT (8)	78	m	5.13	−28.8	18.45	−29.0	10.39	−12.3	33.96	−24.6
SDAT (9)	78	f	0.98	−86.4	6.21	−76.1	10.93	−7.8	18.10	−59.8
SDAT (11)	85	f	2.34	−67.5	27.79	+6.9	13.35	+12.7	43.46	−3.5
PD (12)	76	f	5.17	−28.2	15.72	−39.5	10.23	−13.7	31.11	−30.9
PD (13)	85	f	2.13	−70.4	7.87	−69.7	2.63	−77.8	12.63	−72.0
PD (14)	83	f	2.18	−69.7	9.90	−61.9	11.19	−5.6	23.27	−48.3
PD (15)	85	m	4.40	−38.9	17.66	−32.1	10.26	−13.4	32.31	−28.2
PD (16)	90	m	3.05	−57.6	19.27	−25.9	10.84	−8.5	33.14	−26.4
PD/Dopa – (17)	79	m	0.68	−90.6	1.13	−95.7	0.72	−93.9	2.53	−94.4
PD/Dopa (18)	82	m	5.27	−26.8	9.92	−61.8	3.90	−67.1	19.08	−57.6

TABLE 6. Contribution of Cells of the Different Morphological Classes to the Total Counted Cell Population in the Rostral, Middle, and Caudal Regions of the Loci Coerulei of Both Sides of the Brainstem in Three Age-Matched Control Cases (Ctr), Three Cases of SDAT, and Two Cases of Parkinson's Disease, One Without Dementia (P – D) and One With Dementia, L-Dopa Nonresponsive (P + D/Dopa –)

	Case #	Age	Sex	% LM	% LB	% SM	% SB
Ctrol							
Ctr	1	78	f	8.6	7.3	57.1	26.9
Ctr	2	79	m	8.2	5.6	48.3	37.9
Ctr	3	83	f	6.1	5.3	64.0	24.6
SDAT	4	71	f	31.8	12.1	30.3	25.8
SDAT	8	78	m	7.7	8.2	52.9	31.2
SDAT	9	78	f	8.2	4.6	67.0	20.1
PD	12	76	f	4.6	2.1	82.2	11.1
P+D/dopa –	18	82	m	6.7	4.6	68.2	20.5
Idio							
Ctr	1	78	f	2.5	2.5	55.3	39.8
Ctr	2	79	m	1.6	1.3	42.6	54.6
Ctr	3	83	f	1.2	3.0	43.1	52.7
SDAT	4	71	f	16.4	9.8	50.1	23.7
SDAT	8	78	m	5.7	6.3	39.4	48.6
SDAT	9	78	f	5.6	5.3	63.7	25.4
PD	12	76	f	1.9	1.1	79.7	17.2
P+D/dopa –	18	82	m	4.5	4.6	53.1	37.9
Axial							
Ctr	1	78	f	1.0	2.5	30.9	65.6
Ctr	2	79	m	0.7	1.7	31.6	66.0
Ctr	3	83	f	0.8	2.3	24.3	72.6
SDAT	4	71	f	13.6	13.5	42.6	30.3
SDAT	8	78	m	6.2	7.4	28.0	58.4
SDAT	9	78	f	2.8	6.2	40.2	50.8
PD	12	76	f	1.8	1.5	67.5	29.2
P+D/dopa –	18	82	m	2.8	7.7	43.5	46.0

[1] Large multipolar, LM; large "bipolar," LB; small multipolar, SM; small "bipolar," SB.

Fig. 8. Individual TH-immunoreactive neurons of the LC in PD. Atypical morphology of the four cell types (see text) is barely recognizable. Cell bodies are swollen and plump and dendrites extremely thin and short. a: Large multipolar (LM). b: Large "bipolar" (LB; arrow). c: Small multipolar (SM). d: Small "bipolar" (SB; arrow) neuron. PAP method. ×450.

Fig. 9. Groups of TH-immunoreactive neurons in the LC in PD. a–c: Typical alterations of neuronal morphology (see text), cell remnants, extraneuronal pigment in the neuropil surrounding the neurons. d: Mildly TH-immunoreactive neuron within the LC in PD with Lewy body inclusions (arrows). PAP method. a–c, ×450. d, ×950.

entire nuclei. In all cases, especially in the rostral parts of the LCs, the number of pigmented, nonimmunoreactive neurons was higher than in controls. Cell loss was apparent in all cases but appeared to differ regionally within individual cases as well as between cases. In areas of decreased neuron densities a reduction in the dendritic network in the locus area was also observed.

In both P – D cases (#12 and 13) a high degree of neuronal degeneration was apparent. The cell densities were moderately reduced from normal in case #12 and extremely reduced in case #13. The computer plots of sections from the different LC parts of case #12 (Fig. 10a–c) showed that the general morphological characteristics of the different LC parts were still recognizable. Low-magnification camera lucida drawings of the neurons documented that the visual impression of the altered LC morphology compared to the age-matched control was due to the reduced dendritic arborization (Fig. 10d–f).

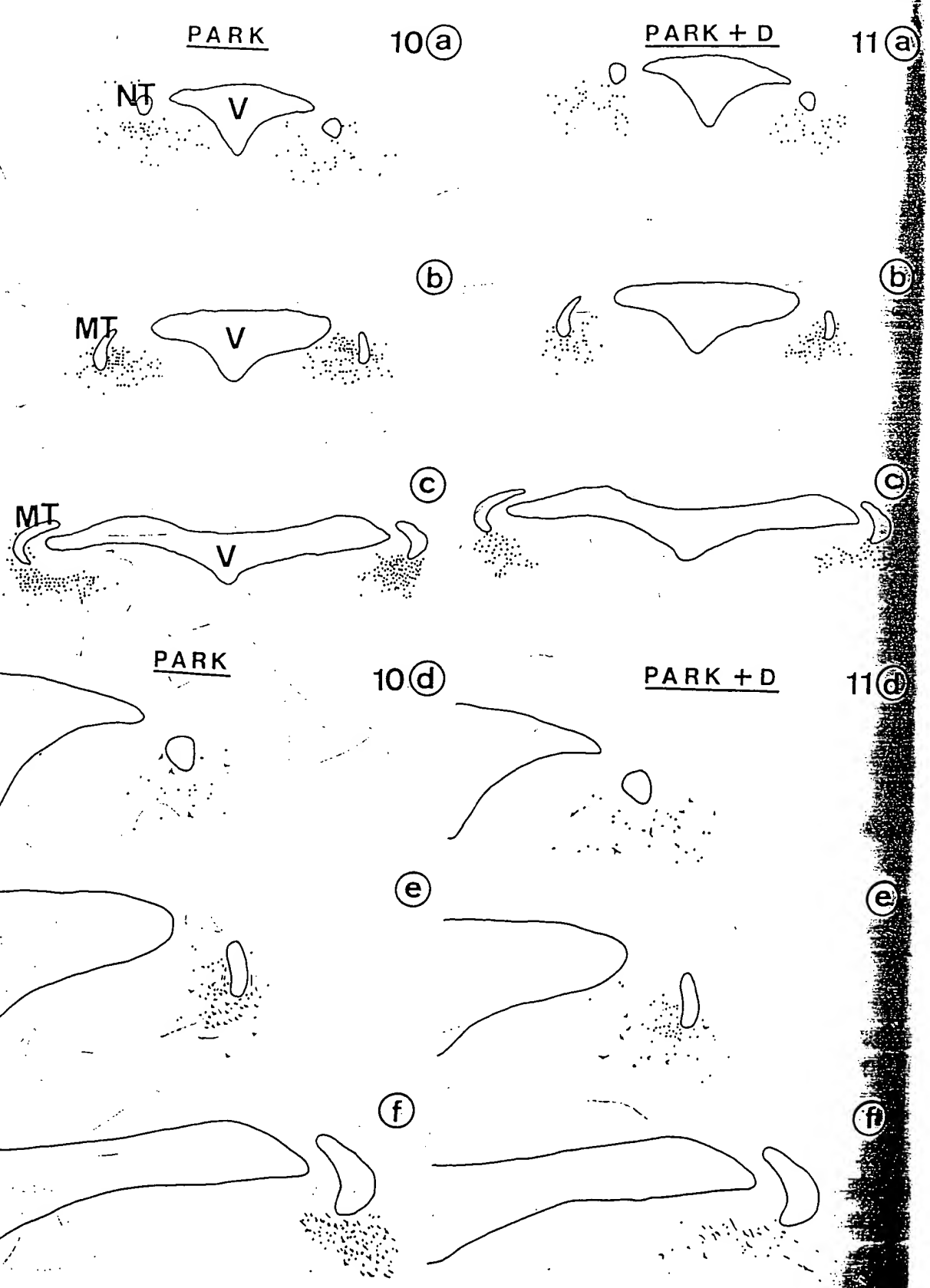
In the P + D cases, the degree of alteration also varied considerably. Case #14 showed severe cell degeneration throughout the LC, whereas in cases #15 and 16 mainly the rostral parts were affected by neuron degeneration and showed more extraneuronal pigment than observed in the controls. In all three cases, cell densities were reduced especially in the rostral and middle parts.

The two cases of L-dopa nonresponsive P + D (cases #17 and 18) showed extreme neuronal alterations. While neuron loss was moderate in case #18, with the greatest reduction in cell density caudally (Figs. 5m–o, 11a–c, and 11d–f), few neurons remained in the loci of case #17.

Axonal terminal fields. In the severely destroyed loci of the majority of cases of PD it was not possible to recognize any TH-immunoreactive axonal elements. In those cases where the morphology was less altered, TH-immunoreactive axons and axon terminals could be identified only with difficulty and in fragments, and, where recognized, were generally fewer than in controls.

Quantitative analyses

Total length of the LC nucleus. On the average, the LCs in the PD cases were shorter than in controls (12.4 and 14.9 mm respectively; Table 4). The standard deviations were almost equally high (1.5 and 1.4 respectively). This suggests that the interindividual variations in length were not as great as in SDAT cases and that in all Parkinson cases the



Figures 10 and 11

lengths were shorter than in the controls. Differences in length between left and right LCs were insignificant (mean difference 0.7% compared to 2.9% in controls).

Neuron numbers. Counts of all TH-immunoreactive LC neurons in both sides of the brainstem were done in four of the PD cases (cases #12, 15, 16, and 18). In cases #13, 14, and 17, in which the LC was damaged on one side due to technical difficulties, an estimation of total cell numbers was made on the basis of the cell counts of the locus of the other side and the cell counts in previous or following serial sections (see Materials and Methods). In these cases, laterality differences between neuron numbers of left and right LCs were not calculated. The approximate cell loss for all cases compared to the average of three age-matched controls was then calculated (Table 5). The impression of cell loss in all cases was verified, ranging from 26.4% to 94.4% (Table 5). Differences in neuron numbers between right and left LCs of those cases in which both LCs were counted were somewhat higher than in controls (1.0–6.6%), ranging from 7.5% to 11.9%. The two Parkinson cases without dementia (P–D) displayed very different results. One case (case #12) presented moderate cell loss (30.9%) with moderate reduction rostrally (28.2%), more in the middle (39.5%), and less caudally (13.7%). The other P–D case (case #13) showed considerable loss (72%) with almost equally high reductions in all regions (70.4%, 69.7%, and 77.8% in rostral, middle, and caudal parts respectively). In all three Parkinson with dementia (P+D) cases (cases #14, 15 and 16), cell loss was moderate to medium high (26.4–48.3%). Reduction was higher rostrally (38.9–69.7%) than in the middle (25.9–49.9%), and lower caudally (5.6–13.4%). In the two Parkinson with dementia cases that were nonresponsive to L-dopa treatment (cases #17 and 18), cell loss was considerable to extreme (57.6% and 94.4%). In case #18 cell loss was highest caudally (67.1%), less in the middle (61.8%), and moderate rostrally (26.8%). Reduction was almost equally high in all regions in case #17 (90.6%, 95.7%, and 93.9% in rostral, middle, and caudal parts respectively).

In two of the seven cases (cases #12 and 18), cell differentiations were carried out (Table 6). The percent contribution of large and small cells appeared to be similar to those in controls in case #12 and in the rostral part of case #18, while in the middle and caudal parts of case #18 the relative number of large cells was higher than in controls. Because of the degree of cell destruction, however, we hold the numbers and relative counts of the different cell classes as tentative because of the difficulty in recognizing cell form in end-stage disease. Alterations in the disease, especially in the cases of small cells, were indicated in case #12 by the relatively large number of small multipolar, i.e., round cells throughout the LC of this case. Many of the small round cells showed signs of degeneration, i.e., loss of dendrites, Lewy bodies, and blurred or irregular somatic outlines.

Three dimensional reconstructions

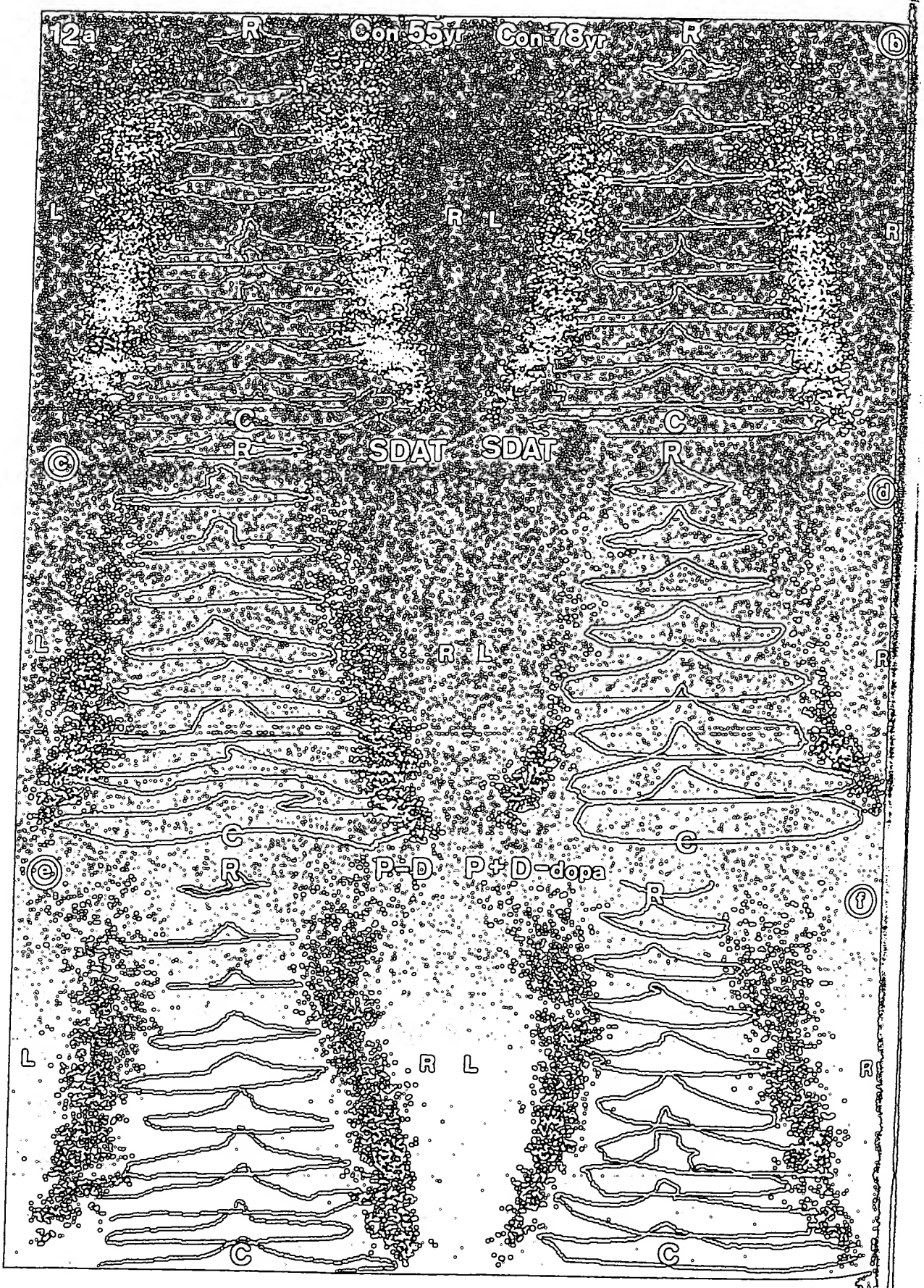
Neuronal distribution (Fig. 12a–f). The three-dimensional reconstructions with dots differently coloured representing the neurons of the four morphological classes (LM: magenta; LB: green; SM: red; SB: yellow) facilitated visual presentation of the characteristics of the LCs of each case studied. Reconstructions of the three-dimensional distribution of LC cells of one young control (Fig. 12a) and one older, age-matched control (Fig. 12b); two paradigm SDAT cases, one mild (Fig. 12c) and one severe (Fig. 12d); and two Parkinson cases, one without dementia (Fig. 12e) and one with dementia, L-dopa nonresponsive (Fig. 12f), illustrated the typical alterations as we have described in the text. The details given in the previous paper (Chan-Palay and Asan, '89) on the reduced neuron densities in the rostral and middle parts of the LCs of the older adult control brain (case #1; Fig. 12b) compared to the younger (Fig. 12a) were seen in the reduction in density and the change of colour of dots. In the two SDAT cases (case #7, Fig. 12c; case #9, Fig. 12d) the LCs of both sides were decreased in length, with the rostral end of the nucleus being notably shortened. Cell density was reduced throughout the locus, most notably in the rostral and middle parts. The thinning out of cells represented by the reduction in dots was most obvious rostrally in the large cells coded magenta. In the Parkinson case without dementia (case #12, Fig. 12e), cell density was only moderately reduced, but the predominance of small multipolar cells was indicated by the colour change to a predominance of red throughout the LC. In the Parkinson case with dementia, L-dopa-nonresponsive (case #18, Fig. 12f), reduction in cell density was obvious, occurring mainly caudally.

Solid reconstruction (Fig. 13a,b). The solid model of the LCs (green and red) of a control (case #1, Fig. 13a) with the fourth ventricle (white) was to be compared with that of an SDAT case (case #7, Fig. 13b). The alterations of the rostral and middle regions, in particular shrinkage and foreshortening, of the LCs of both sides (green and red) in the SDAT case were well illustrated. While the ventricular space was widened in the SDAT case, the lengths and areas of the entire LCs were considerably reduced, thus leading to a shrunken volume of the LCs of left (green) and right (red) sides compared to the control.

DISCUSSION

The present study sought to provide detailed information about alterations occurring in the brainstem nucleus locus coeruleus in two neurodegenerative diseases involving dementia—the senile dementia of the Alzheimer type (SDAT) and Parkinson's disease (PD). In order to properly document the relationships between the LC-NE system and the degree and specific type of dementia, the cases in this study had been closely observed during prolonged hospitalization. The cognitive performance of each patient had been previously assessed in psychometric tests. We have distinguished three groups of PD patients: one without features of dementia, one with dementia in which the patient's motor symptoms responded well to L-dopa treatment, and one in which in addition to motor disorders nonresponsive to L-dopa treatment, a fulminant progressive dementia was a prominent feature.

s. 10, 11. The distribution of TH-immunoreactive LC neurons in l (a,d), middle (b,e), and caudal (c,f) parts of a PD case without ntia (Fig. 10a–f) and one with dementia, L-dopa nonresponsive 11a–f). The plots from Figures 10a–c and 11a–c and the camera drawings from Figures 10d–f and 11d–f were done as described in gend of Figures 6 and 7, and the outlines of the fourth ventricle ne trochlear nerve (NT), and the mesencephalic trigeminal tract are marked in Figure 10a–c. Compare also to the plots and camera drawings of the control case (Fig. 6) and the SDAT case (Fig. 7). hat the cell densities are reduced from normal mainly rostrally the middle in the PD case without dementia, and in all parts of in the PD case with dementia, L-dopa nonresponsive. The neulegeneration present throughout the LCs in both PD cases is most s in the camera lucida drawings.


12a
R
Con 55yr
Con 78yr
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b
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Figure 12

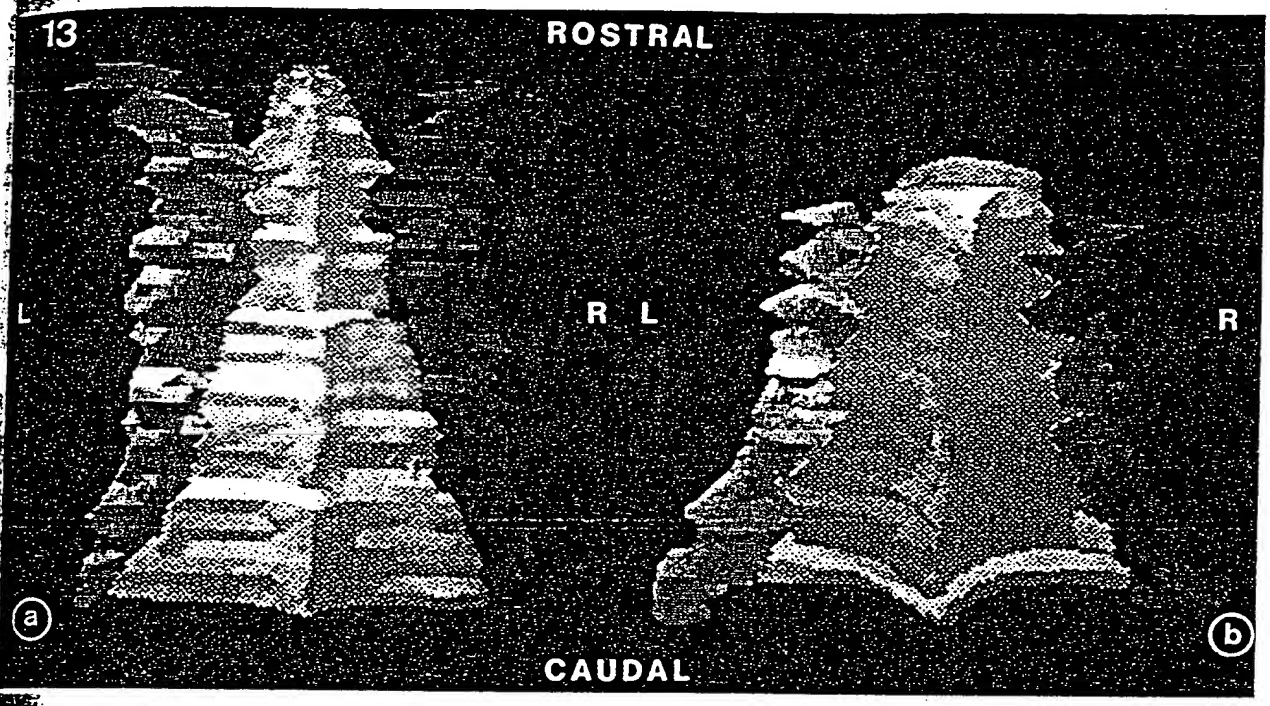

Fig. 13. Three-dimensional computer reconstruction in solid form of the fourth ventricle and the loci of left and right LC in (a) an older control case, 78 year, case #1, and (b) an SDAT case, 77 year, case #7. The reconstruction is viewed from dorsal, shifted in a 25° angle from the plane of the figure. Orientations rostral and caudal and left and right are defined. The green colour denotes the solid reconstruction of the LC on the left and the red the LC on the right brainstem side. The ventricle is shown in semitransparent white. The changes in SDAT, i.e., shorter nuclear length, cell loss particularly in the rostral part, smaller LC, and enlarged ventricle, are clearly documented.

The LC in SDAT

The reduction of neuron numbers, total LC length, and morphological changes of individual neurons ranged from moderate to extreme in the different cases. In the classical SDAT cases characterised by clinicians and neuropathologists (#4, 7, 9) the pattern of typical alterations included a severe alteration in individual neuron morphology, suggesting a defect in cytoskeletal integrity. This is in accordance with the findings of neuritic disruption due to abnormal cytoskeletal proteins demonstrated with monoclonal antibodies both in neurons containing neurofibrillary tangles and in nontangle-bearing neurons (Selkoe, '87). These alterations are present generally throughout the nucleus, but particularly rostrally. Nuclear length is reduced because of the foreshortening at the rostral tip. Extraneuronal pigment and extremely weakly immunoreactive pigmented cells are more numerous than in age-matched controls, and neuron numbers are reduced. A topographical arrangement of cell loss is evident in the LC, affecting rostral, middle, and caudal parts by order of decreasing intensity (Lockhart et al.,

Fig. 12. Three-dimensional computer reconstruction in colour of the LC of (a) a younger control case, 55 year (for full description see text Tan-Palay and Asan, '89); (b) an older control case, 78 year, case #1; (c) a case of mild SDAT with comparatively little cell loss, 78 year, case #2; (d) a severe SDAT case with extreme cell loss, 77 year, case #7; (e) a PD case without dementia, 76 year, case #12; and (f) a PD case with dementia, L-dopa nonresponsive, 82 year, case #18. The reconstruction is viewed from dorsal, shifted in a 25° angle from the plane of the figure. R and C on the top and bottom of each figure signify the rostral and caudal tip of the LC and R and L on the sides the right and left LC. The outline of the fourth ventricle (blue) is drawn on every fourth section, and each TH-immunoreactive neuron on all the recorded sections is marked by a coloured dot, the colours signifying the different morphological classes: LM: magenta; LB: green; SM: red; SB: yellow. Note the cell loss which occurs mainly in the rostral part of the older control case (b) compared to the younger control case (a). Note also the high neuronal loss present predominantly in the rostral and middle parts in both SDAT cases c and d. In the PD case with dementia/L-dopa nonresponsive (f) cell loss is present throughout the nucleus. The colour change to red predominance due to the presence of many small round (multipolar SM) cells is most obvious in the PD case without dementia (e).

TABLE 7. Summary of the Number (1×10^3) of TH-Immunoreactive Neurons and % Loss of Neurons in Loci Coerulei of Age-Matched Controls, Patients With Alzheimer's Dementia, and Parkinson's Disease Without and With Dementia, and With Dementia, L-Dopa Nonresponsive

Case	Age	Sex	Neuron no.	% neuron loss
Control	79	m	47.5	
Control	78	f	40.9	
SDAT	78	m	34.0	−24.6
SDAT	74	f	18.8	−58.2
SDAT	77	f	5.7	−87.5
P−D	76	f	31.1	−30.9
P+D	83	f	23.3	−48.3
P+D/L-dopa non-resp.	79	m	2.5	−94.4

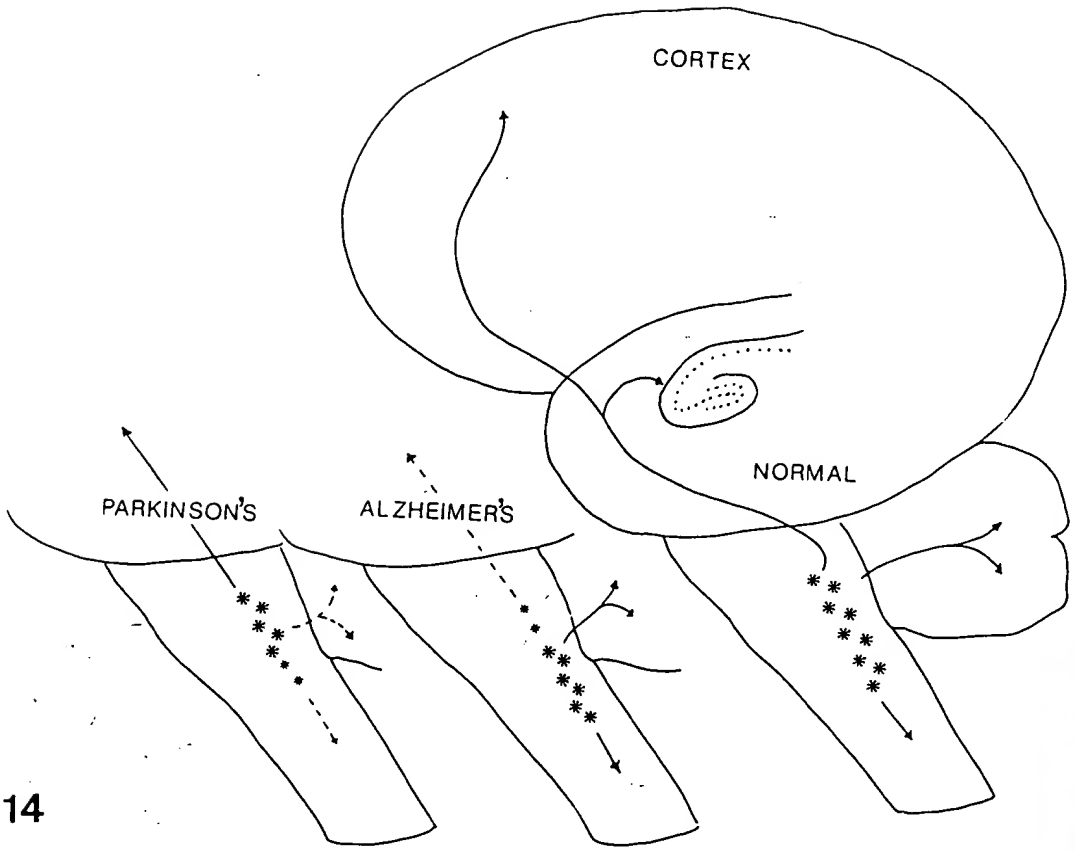

Fig. 14. Summary diagram to illustrate the changes in LC catecholamine neurons in PD and SDAT in comparison to the age-matched control. In SDAT the neuron loss is rostrally, thereby involving the projections of these neurons to the hippocampus and neocortex. In PD the neuron alterations and loss are greater in the middle and caudally, leaving the neocortical projections from rostrally less affected than the cerebellum and spinal cord.

'84; Marcyniuk et al., '86; Ingram et al., '87a,b). In the majority of cases, laterality differences in neuron numbers are higher than in controls. Since the cells remaining are larger in somatic size in the middle and caudal parts than in controls, we surmise that the individual neurons undergo soma swelling and arbor reduction; this may be due to the disintegration of the cytoskeleton, or to a chromatic reaction due to a loss of axons in the cortical projection areas. If the topographical organisation of the LC postulated for animals (Mason and Fibiger, '79; Loughlin et al., '82) holds true also for humans, then the variation of alteration in the different parts of the LC may suggest that there may be a correlation with neuropathological changes in the projection area of these neurons in the neocortex. In a recent study it has been demonstrated that some LC neurons in the human contain neuropeptides (Chan-Palay et al., '89), which seem to survive better in SDAT, thus suggesting a differential susceptibility or preservation of LC neurons.

The LC in Parkinson's disease

The degree of LC degeneration was found to be extremely variable, both within the Parkinson's group as a whole and within each of the subgroups. However, some features common to most of the cases could also be recognized. LC neuron loss and degeneration are severe, with the occurrence of Lewy inclusions and extraneuronal pigment, swelling of cell somata and loss of dendritic arbors, irregular immunoreactions, and blurred cell outlines; the changes are more severe than in SDAT. Because of the neuronal degeneration previous in most cases, categorization of neurons into the different morphological classes was difficult. Generally, cells basically recognizable as small cells tend to be round and denuded of dendrites and axons. In areas of cell loss the contribution of large cells is greater than normal. The length of the LC is reduced, and the alterations detailed occur in most cases rostrally, and in most cases throughout the entire

ucleus. In PD cases, the middle and caudal parts are more severely affected than in SDAT. Interestingly, there are differences in the degree of regional neuronal loss within the subgroups. P+D cases have greater loss rostrally than in the middle part and are almost entirely spared from reduction caudally. In the P+D/L-dopa nonresponsive cases, the caudal part is severely affected with better preservation rostrally. The two P−D cases differ considerably in their comparative degree of cell loss; caudally, reduction of neuron numbers is extreme in one and almost negligible in the other case. Both display considerable cell loss in the middle part compared to rostrally.

The findings and conclusions of this study are summarized in Table 7 and Figure 14. In SDAT and PD, catecholamine neuron numbers in the LCs of both sides are reduced. These reductions are topographically arranged. In the brains of SDAT cases the loss is greatest rostrally. In the PD group of patients, the catecholamine neurons of the LC are more severely affected in the middle and caudally than in SDAT. In the demented, L-dopa nonresponsive PD patients, cell loss is high overall in the LC, but particularly so caudally. The lowest count of catecholamine LC neurons was encountered in a patient in the P+D/L-dopa nonresponsive category. Assuming that a topographical arrangement of LC neurons exists in man as has been reported for experimental animals (Mason and Fibiger, '79; Loughlin et al., '82), then the neuron loss and/or destruction in the rostral parts of the LC in SDAT and P+D might suggest a significant loss in neocortical and hippocampal NE innervation. The loss in the caudal parts of the LC might suggest a reduced NE innervation in the spinal cord, the cerebellum, and areas other than the neocortex. Severe overall reduction in P+D/L-dopa nonresponsive cases suggests impairment in all regions innervated by the LC neurons, thus supporting the observation that these cases suffer from a fulminant rapidly progressive dementia. The LC changes that we report here may not be the primary cause of the cognitive disorders but may explain the clinical symptomatology, i.e., lack of alertness and vigilance, functions found to be especially reduced in PD patients. Moreover, we have reported significant LC cell loss in depression without dementia (-34.3%, Chan-Palay and Asan, '89). This is comparable to the cell losses encountered in mild to severe cases of SDAT and in cases of Parkinson's disease with and without dementia. The presence of depression in SDAT and Parkinson's with dementia patients is accompanied by the greatest loss of LC neurons (see also Ross et al., '87, for depression in SDAT).

These immunocytochemical findings allow us to report: changes in LC catecholamine neuron morphology; the topographic distribution of the neurons with these alterations; and the number of neurons destroyed in each dementing disease which clearly differentiate between the controls, SDAT cases, and those in the Parkinson's disease group. The changes are consistent enough to suggest diagnoses of these conditions on the basis of the TH-immunoreactive material. We have since applied this new capability to diagnostic use in succeeding postmortem cases. In combination with clinical information, psychometric test results, and macro- and microneuropathology this additional diagnostic tool based on TH immunocytochemistry has clarified the postmortem decision of diagnosis for several difficult cases, especially those involving a mixture of SDAT and PD symptomatology.

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